Geographical difference of disease association in *Streptococcus bovis* bacteraemia

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From 1996 to 2001, 48 *Streptococcus bovis* strains were isolated from blood cultures of 37 patients in one hospital. Median patient age was 68 years (range: 1 day–88 years). The male : female ratio was 23 : 14. Most patients (97 %) had underlying diseases, including biliary tract disease in 14 (38 %), diabetes mellitus in 12 (32 %), liver parenchymal disease in seven (19 %), carcinoma of the colon in four (11 %) and other malignancies in four (11 %). No infective foci (indicative of primary bacteraemia) were identified in 15 patients (40 %) and 14 (38 %) had acute cholangitis/cholecystitis, but only four (11 %) had infective endocarditis. Two (5 %), three (8 %) and 32 (87 %) patients had *S. bovis* of biotypes I, II/1 and II/2, respectively, and three (8 %), two (5 %) and 32 (87 %) patients had *S. bovis* of genotypes 1, 2a and 2b, respectively. All isolates were sensitive to penicillin, cephalothin and vancomycin, 24 (65 %) were resistant to erythromycin and 15 (41 %) were resistant to clindamycin (these strains were also resistant to erythromycin). Thirteen isolates that were erythromycin- and clindamycin-resistant possessed the *ermB* gene, 10 possessed the *ermT* gene and one possessed both the *ermB* and *ermT* genes. Overall, seven patients (19 %) died. In contrast to most other reports from western countries, where carcinoma of the colon and infective endocarditis were the major underlying disease and infective focus associated with *S. bovis* bacteraemia, biliary tract disease and acute cholangitis and/or cholecystitis were the major underlying diseases associated with *S. bovis* bacteraemia in our locality.

Received 29 January 2003
Accepted 1 July 2003

INTRODUCTION

Streptococcus bovis is frequently recovered as a commensal in both humans and animals. Isolation of *S. bovis* from blood cultures of patients has a documented association with carcinoma of the colon and infective endocarditis (Klein *et al.*, 1977, 1979; Steinberg & Naggar, 1977; Murray & Roberts, 1978; Reynolds *et al.*, 1983; Kupferwasser *et al.*, 1998). Two reports have also demonstrated an association of *S. bovis* bacteraemia with chronic liver parenchymal disease (Zarkin *et al.*, 1990; Gonzalez-Quintela *et al.*, 2001). Phenotypically, the API 20 STREP system (bioMérieux) subdivides *S. bovis* into three distinct biotypes, namely I, II/1 and II/2 (Ruoff *et al.*, 1989). Many reports have identified *S. bovis* biotype I as the predominant isolate in cases of bacteraemia caused by the *S. bovis* group (Murray *et al.*, 1999; Songy *et al.*, 2002); it is also the biotype that is particularly associated with carcinoma of the colon and infective endocarditis (Murray *et al.*, 1999). Genotypic studies that used DNA sequencing of a 500 bp fragment of the 16S rRNA gene have shown that there is concordance between different biotypes and genotypes (Clarridge *et al.*, 2001). Recently, based on a combination of DNA homology studies, whole-cell protein analysis

and sequencing of the *sodA* gene, suggestions have been made for the revision of *S. bovis* taxonomy. *Streptococcus gallolyticus*, *Streptococcus infantarius* and *Streptococcus pasteurianus* have been proposed to replace *S. bovis* I, *S. bovis* II/1 and *S. bovis* II/2, respectively (Facklam, 2002).

One study showed a high incidence of erythromycin resistance in *S. bovis* (Teng *et al.*, 2001). Despite in-depth phenotypic and genotypic characterization and disease association studies in western countries, none have attempted to study the epidemiology and disease association of *S. bovis* bacteraemia in countries outside North America and Europe.

In this study, we characterized 48 *S. bovis* strains that were isolated from blood cultures of 37 bacteraemic patients over a 6-year period by using a combination of phenotypic and genotypic techniques. Epidemiology, underlying diseases, clinical disease associations and outcome of *S. bovis* bacteraemia in relation to the different biotypes and genotypes of *S. bovis* and molecular epidemiology of erythromycin resistance in *S. bovis* strains were investigated.

METHODS

Patients and microbiological methods. The 37 patients in this study were hospitalized at the Queen Mary Hospital in Hong Kong during a 6-year period (1996–2001). All clinical data were collected as described previously (Woo *et al.*, 2001c). Suspect colonies were identified by standard conventional biochemical methods (Murray *et al.*, 1999). In addition, the API 20 STREP system (bioMérieux) was used for identification of all *Streptococcus*-like (*Streptococcus*, *Enterococcus*, *Granulicatella*, *Abiotrophia* and *Gemella*) isolates and biotyping of *S. bovis* isolates. Antimicrobial susceptibility was tested by the Kirby–Bauer disc-diffusion method and results were interpreted according to NCCLS criteria (Bauer *et al.*, 1966).

Extraction of bacterial DNA. Bacterial DNA extraction was modified from our previously published protocol (Woo *et al.*, 1997). In brief, 80 µl NaOH (0.05 M) was added to 20 µl bacterial cells suspended in distilled water and the mixture was incubated at 60 °C for 45 min, followed by addition of 6 µl Tris/HCl (pH 7.0) to achieve a final pH of 8.0. The resultant mixture was diluted 100× and 5 µl diluted extract was used for PCR.

Genotyping by 16S rRNA gene sequencing. PCR amplification and DNA sequencing of a 500 bp fragment of the 16S rRNA gene were performed as described previously (Woo *et al.*, 2000, 2001a, b, 2002a, b, c; Yuen *et al.*, 2001; Lau *et al.*, 2002) by using primers LPW601 (5'-ATGGGAGAGTTTGATCCCTG-3') and LPW602 (5'-TACCCG CGGCTGCTGGCACG-3') (Gibco-BRL). Sequences of PCR products were compared with known 16S rRNA gene sequences in GenBank by multiple sequence alignment with the CLUSTAL W program (Thompson *et al.*, 1994) and phylogenetic tree construction was performed by using PileUp with GrowTree (Genetics Computer Group).

erm gene sequencing. PCR amplification and DNA sequencing of the *erm* genes were performed according to a previously published method (Teng *et al.*, 2001) by using primers LPW632 (5'-GAAAAGGTACT CAACCAAATA-3') and LPW633 (5'-AGTAACGGTACTTAAATT GTTTAC-3') for the *ermB* gene and LPW636 (5'-GCTAATATTGTT TAAATCGTCAATTCC-3') and LPW637 (5'-GGATCAGGAAAAG GACATTTTAC-3') for the *ermT* gene (Gibco-BRL). Sequences of PCR products were compared with known *erm* gene sequences in GenBank by multiple sequence alignment with the CLUSTAL W program (Thompson *et al.*, 1994) and phylogenetic tree construction was performed by using PileUp with GrowTree (Genetics Computer Group).

RESULTS AND DISCUSSION

In a 6-year period (1996–2001), 48 strains of *S. bovis* were isolated from blood cultures of 37 patients and were identified as *S. bovis* by the API 20 STREP system. *S. bovis* strains recovered from the same patient exhibited the same biotype and genotype based on 16S rRNA gene sequence analysis. Characteristics of the 37 patients with *S. bovis* bacteraemia were as follows: median age was 68 years (range: 1 day–88 years) and 23 patients (63 %) were over 60 years of age. The male : female ratio was 23 : 14. Most patients (97 %) had underlying diseases: the major underlying diseases included biliary tract disease in 14 (38 %), diabetes mellitus in 12 (32 %), liver parenchymal disease in seven (19 %), carcinoma of the colon in four (11 %) and other malignancies in four (11 %). The only patient without underlying disease was a 2-year-old boy with gastroenteritis; the *S. bovis* strain in this patient's blood had probably translocated from

the gastrointestinal tract as a result of intestinal inflammation. No infective foci (indicative of primary bacteraemia) were identified in 15 patients (40 %), whereas 14 (38 %) had acute cholangitis/cholecystitis, four (11 %) had infective endocarditis, one (3 %) had secondary peritonitis due to a perforated appendix, one (3 %) had neonatal meningitis, one (3 %) had spontaneous bacterial peritonitis and one (3 %) had cellulitis. All patients had community-acquired *S. bovis* bacteraemia. *S. bovis* was recovered from a single blood culture from 32 patients (87 %), whereas the organism was isolated from multiple blood cultures from five patients (13 %). Two (5 %), three (8 %) and 32 (87 %) patients had *S. bovis* of biotypes I, II/1 and II/2, respectively. Three (8 %), two (5 %) and 32 (87 %) patients had *S. bovis* of genotypes 1, 2a and 2b, respectively (Fig. 1). *S. bovis* was the only bacterium recovered from blood cultures of 25 patients

Fig. 1. Phylogenetic tree showing the relationship of *S. bovis* strains recovered from 37 patients. The tree was inferred from 500 bp of the 16S rRNA gene sequence by the neighbour-joining method. Names and accession numbers are given as cited in GenBank. Bar, 1 substitution per 100 bases (estimated by using the Jukes–Cantor correction).

(68 %) whereas in 12 patients (32 %), other bacteria were recovered concomitantly with *S. bovis* from blood cultures (*Escherichia coli* in five patients, *Citrobacter farmeri* in one patient, *Klebsiella pneumoniae* in one patient, *Escherichia coli* and *K. pneumoniae* in two patients, *Escherichia coli* and *Enterococcus faecalis* in one patient, *Escherichia coli*, *Bacteroides fragilis* and *Fusobacterium* sp. in one patient and *Escherichia coli*, *K. pneumoniae* and *Aeromonas hydrophila* in one patient). Only one patient, with neonatal meningitis, had *S. bovis* recovered at other sites in addition to blood (cerebrospinal fluid and nasal swab). Isolates recovered from all patients were sensitive to penicillin, cephalothin and vancomycin, whereas those recovered from 24 patients (65 %) were resistant to erythromycin and those recovered from 15 patients (41 %) were resistant to clindamycin (these strains were also resistant to erythromycin). Overall, seven patients (19 %) died.

The *erm* genes of the 24 *S. bovis* isolates that were resistant to erythromycin were amplified and sequenced. Thirteen iso-

lates that were erythromycin- and clindamycin-resistant possessed the *ermB* gene, 10 possessed the *ermT* gene and one possessed both the *ermB* and *ermT* genes (Fig. 2).

In this study, we describe the characterization of 48 *S. bovis* strains isolated from 37 patients with *S. bovis* bacteraemia. Similarly to a recent study that described the characterization of 22 clinical strains of *S. bovis* (Clarridge *et al.*, 2001), we also demonstrated concordance between the different biotypes (as identified by the API 20 STREP system) and genotypes (based on 16S rRNA gene sequencing). Similarly to this recent study and another in Taiwan (in which 88 % of 60 blood culture isolates were of biotype II/2) (Teng *et al.*, 2001), biotype II/2 or genotype 2b was the most predominant type of *S. bovis* found in our patients (Table 1). This is different from the findings of previous studies that demonstrated that biotype I was the predominant type of *S. bovis* isolated from their bacteraemic patients (Ruoff *et al.*, 1989).

In contrast to previous studies that described a strong association between *S. bovis* bacteraemia and carcinoma of

Fig. 2. Phylogenetic tree showing the relationship of the amino acid sequences of the *ermB* and *ermT* genes of the 24 erythromycin-resistant *S. bovis* strains to those of other *erm* genes. The tree was inferred from 174 aa sequence data by the neighbour-joining method. Names and accession numbers are given as cited in GenBank. Bar, 5 substitutions per 100 amino acids (estimated by using the Kimura correction).

Table 1. Comparison of characteristics from major studies of *Streptococcus bovis* bacteraemia

References: 1, Murray & Roberts, 1978; 2, Reynolds *et al.*, 1983; 3, Pigrau *et al.*, 1988; 4, Ruoff *et al.*, 1989; 5, Zarkin *et al.*, 1990; 6, Clarridge *et al.*, 2001; 7, Gonzalez-Quintela *et al.*, 2001; 8, Teng *et al.*, 2001; 9, present study. Unless stated, data are number of patients with the characteristic, with percentage in parentheses. NM, Not mentioned.

Characteristic	Major studies on <i>S. bovis</i> bacteraemia								
	1	2	3	4	5	6	7	8	9
No. patients	36	19	16	38	92	12	20	60	37
Mean age (years)	61	NM	49	67	57	NM	62	NM	61
Sex:									
Male	19 (53)	11 (58)	8 (50)	21 (55)	45 (49)	NM	13 (65)	NM	23 (62)
Female	17 (47)	8 (42)	8 (50)	17 (45)	47 (51)	NM	7 (35)	NM	14 (38)
Underlying diseases:									
Liver parenchymal disease	0 ^{b*} (0)	0 ^a (0)	4 (25)	NM	45 ^c (47)	0 (0)	11 ^b (55)	NM	7 (19)
Biliary tract disease	2 ^d (6)	0 ^d (0)	3 (19)	NM	3 ^d (3)	0 ^b (0)	0 ^d (0)	NM	14 (38)
Colonic carcinoma	5 (14)	2 (11)	1 (6)	15 ^c (40)	16 (17)	1 (8)	6 (30)	NM	4 (11)
Benign lower gastrointestinal tract diseases	10 ^c (28)	11 ^d (58)	NM	NM	6 (7)	NM	0 (0)	NM	1 (3)
Diagnosis:									
Primary bacteraemia	7 ^a (19)	2 ^a (11)	3 (19)	NM	56 ^a (61)	6 (50)	NM	NM	15 (41)
Cholangitis/cholecystitis	2 ^d (6)	0 ^d (0)	3 (19)	NM	3 ^d (3)	0 ^b (0)	0 ^d (0)	NM	14 (38)
Infective endocarditis	26 ^d (72)	17 ^d (47)	5 (31)	19 ^d (50)	26 ^a (28)	4 (33)	10 ^d (50)	NM	4 (11)
Biotypes of <i>S. bovis</i> :									
I	NM	NM	NM	17 ^d (45)	NM	1 (8)	NM	4 (7)	2 (5)
II/1	NM	NM	NM	12 ^a (32)	NM	1 (8)	NM	3 (5)	3 (8)
II/2	NM	NM	NM	9 ^d (24)	NM	10 (83)	NM	53 (88)	32 (87)
Mortality	3 (8)	NM	5 (31)	9 (24)	NM	NM	8 (40)	NM	7 (19)

*Statistically significant when compared to the present study by using a χ^2 test at the following levels: a, $P < 0.05$; b, $P < 0.01$; c, $P < 0.005$; d, $P < 0.001$.

the colon and infective endocarditis (Steinberg & Naggar, 1977; Klein *et al.*, 1977, 1979; Murray & Roberts, 1978; Reynolds *et al.*, 1983; Kupferwasser *et al.*, 1998), the association between *S. bovis* bacteraemia and these clinical conditions was not strong in our population (Table 1). Previous studies have shown that associations between *S. bovis* bacteraemia and carcinoma of the colon and infective endocarditis were even higher for *S. bovis* biotype I isolates, with 94 % association of *S. bovis* biotype I bacteraemia with infective endocarditis and 71 % association of *S. bovis* biotype I bacteraemia with colonic carcinoma (Murray *et al.*, 1999). On the other hand, for *S. bovis* isolates of biotype II, association between bacteraemia and infective endocarditis was only 18 % and that between bacteraemia and carcinoma of the colon was only 17 % (Murray *et al.*, 1999). As our *S. bovis* strains mainly belonged to biotype II/2, it is not surprising that we have found a weak association between *S. bovis* bacteraemia and infective endocarditis and carcinoma of the colon. In the present study, the rate of endocarditis and carcinoma of the colon was only 11 %. Interestingly, none of the four patients with infective endocarditis and the four with carcinoma of the colon had bacteraemia caused by *S. bovis* biotype I.

Biliary tract disease and acute cholangitis and/or cholecystitis

were the predominant underlying diseases and diagnoses associated with *S. bovis* bacteraemia in our locality. An association between *S. bovis* bacteraemia and chronic liver parenchymal disease has been reported previously (Table 1) (Zarkin *et al.*, 1990; Gonzalez-Quintela *et al.*, 2001). Pigrau *et al.* (1988) first noted that four of 16 cases of *S. bovis* bacteraemia had liver cirrhosis; subsequently, in a study by Zarkin *et al.* (1990), evidence of liver parenchymal disease was observed in 45 of 92 patients with *S. bovis* bacteraemia. More recently, Gonzalez-Quintela *et al.* (2001) described 11 of 20 patients with *S. bovis* bacteraemia that had chronic liver parenchymal disease. In the present study, 14 of 37 patients (38 %) had biliary tract disease and the same number of patients had acute cholangitis and/or cholecystitis. We speculate that the association of biliary tract disease with *S. bovis* bacteraemia is due to the following facts: firstly, that biliary tract disease is prevalent in our locality. In one study, it was demonstrated that biliary tract sepsis is the second most common cause of community-acquired bacteraemia after urinary tract infection (French *et al.*, 1990). This uniquely high incidence of biliary sepsis in this part of the world is attributed to high prevalence of clonorchiasis and recurrent pyogenic cholangitis (Lo *et al.*, 1997; Woo *et al.*, 1998). Secondly, in contrast to most α -haemolytic streptococci, *S. bovis* is able to grow in bile (Luk *et al.*, 1998). This property

renders it able to survive in bile and cause acute cholecystitis and cholangitis, especially in the presence of pre-existing biliary tract disease. Therefore, in our locality, biliary tract disease should be actively looked for in any future case of unexplained *S. bovis* bacteraemia.

The *ermB* and *ermT* genes in the *S. bovis* isolates studied are highly homologous to those found in other bacteria of the gastrointestinal tract. Incidence and molecular epidemiology of erythromycin resistance in the present study are similar to those reported in a recent study from Taiwan (Teng *et al.*, 2001). Similarly to the Taiwan study, which reported that 63 % of *S. bovis* bacteraemic isolates were erythromycin-resistant, 65 % of *S. bovis* isolates in our locality were erythromycin-resistant. Furthermore, 58 and 46 % of our erythromycin-resistant isolates contained the *ermB* or *ermT* genes, respectively, which is also similar to the corresponding incidences of 63 and 37 % reported in that study. The *ermB* and *ermT* genes in *S. bovis* showed high homology to the *ermB* genes in *Clostridium difficile*, *Clostridium perfringens*, *Lactobacillus reuteri*, *Streptococcus agalactiae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Lactobacillus fermentum* and *Escherichia coli* and the *ermT* genes in *L. reuteri* and another *Lactobacillus* species, which are all bacteria from the gastrointestinal tract (Fig. 2). We therefore speculate that these genes in *S. bovis* were acquired through horizontal gene transfer from other bacteria that reside in the gastrointestinal tract.

ACKNOWLEDGEMENTS

This work is partly supported by the University Development Fund, University Research Grant Council and the Committee for Research and Conference Grant, The University of Hong Kong.

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